RESEARCH METHOD
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3 - INDOLE BUTYRIC ACID Issue No 2 270686 B-37-2/5

Research Method

COMPLETE CHECK OF SAMPLE NO 1
CHECKING ALL OTHER SAMPLES ON: appearance and identity

Appearance

Point of fusion

Mettler FP 80/81, 1 °C minute

Identity

Adsorbent	Silica gel F 254
Developer	Hexane : Acetone : Acetic Acid 100% (60:40:5)
Range	15 cm
Detection	UV-light 254 mm

Weigh out 100 mg of 3-Indole Butyric Acid. Add 10 ml of Methanol and let dissolve. Apply 5 microlitres of this solution to the plate. Then apply 5 microlitres of the standard solutions to the plate. Examine the plate after developing and drying under UV-light.

Rf 3-Indole Butyric Acid	0.41
Rf 3-Indole Acetic Acid	0.35
Rf 1-Naphthalene Acetic Acid	0.46

Standard 3-Indole Acetic Acid : 100 mg in 10 ml of Methanol
Standard 3-Indole Butyric Acid: 100 mg in 10 ml of Methanol

Iron

Assimilate the remnant obtained from the Sulphate Ashes into 10 ml of water. Add 2 ml of a 20% Citric Acid solution and 0.1 ml of Thio Glycolic Acid. Mix those ingredients, alkalify with Ammonia 20% and replenish with water to 20 ml.
Make a standard solution by identically processing a 10 ml Iron solution (1 ppm Fe).
After five minutes the intensity of the pink colouration of the sample solution may not predominate the colouration of the standard solution.

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Heavy metals

Weigh out 1.00 gram of 3-Indole Butyric Acid into a china crucible. Add 4 ml of a solution of Magnesium Sulphate 25% in 2N Sulphuric Acid. Mix with the aid of a glass stick and heat carefully. Let the mixture evaporate on a water bath after reaching a liquid state. Then burn until a white or grey substance is obtained (800 °C).
Moisten the remnant with a few drops of 2N Sulphuric Acid, then evaporate and burn again and let cool down. Assimilate the remnant with 2 x 5 ml of 2N Hydrochloric Acid.
Add 0.1 ml of a Phenol Phthalic Acid solution and replenish with Ammonia 25% until the solution has achromatized. Subsequently add 0.5 ml of Acetic Acid overmeasure.
Filter if necessary and thin down with water till reaching a volume of 20 ml (i.e. solution A). Add 12 ml of solution A as well as 2 ml of an Acetate buffer (pH 3.5) to 1.2 ml of a Thio Amide reagent and intermix. After 2 minutes the intensity of the brown colouration may not predominate the colouration of the standard solution, which can be obtained by processing a mixture of 4 ml of a solution of Magnesium Sulphate 25% in 2N Sulphuric Acid and 1.0 ml of Lead Nitrate (10 ppm Pb) similar as mentioned above (burn, assimilate with Hydrochloric Acid, etc). Take 10 ml of this solution and add 2 ml of the sample solution (i.e. solution B). Add 12 ml of solution B together with 2 ml of an Acetate buffer (pH 3.5) to 1.2 ml of a Thio Amide reagent and intermix.

Sulphate Ashes

Burn a crucible at 800 °C, let it cool down and determine the weight. Weigh out 1.00 gram of 3-Indole Butyric Acid, moisten this with concentrated Sulphuric Acid and burn again at 800 °C. Repeat moistening with Sulphuric Acid and subsequent burning until maintaining a stable weight.

Drying loss

Shrink 1 gram at 105 °C until maintaining a stable weight.

Particle size

Take a 125 micrometer sieve.
Sift 10 grams of 3-Indole Butyric Acid during 3 minutes. Determine the remnant.

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3-INDOLE BUTYRIC ACID Issue No 2 270686 B-37-4/5

Research Method

Grade of 3-Indole Butyric Acid

Chromatography

Fractionator	:	15 cm x 4.6 mm Hypersil ODS 5 micrometers
Mobile phase	:	0.0025 M Tetra Butyl Ammonium Hydroxide in a mixture of Methanol + 0.1 M Sodium Acetate (21:18) made up to pH 8.0 with Acetic Acid 30% (10.0 ml of 0.1M TBAH / 210 ml of Methanol / 180 ml of 0.1 M Sodium Acetate)
Flow	:	0.6 ml / min
Detection	:	UV 280 nm 0.1 AU
Printer / Plotter	:	AT = 128
Injection	:	10 microlitres
Retention time	:	3-Indole Butyric Acid approx. 4 minutes

Standard: 118715 XF = 99.9

Weigh out approx. 50 mg of 3-Indole Butyric Acid into a 250 ml volumetric flask and determine the weight exactly. Then dissolve in 15 ml of a Methanol / 0.1 N NaOH mixture (2:1). Fill up with a Methanol / water mixture (1:1).

Sample: DUAL

Weigh out approx. 50 mg of 3-Indole Butyric Acid into a 250 ml volumetric flask and determine the weight exactly. Then dissolve in 15 ml of a Methanol / 0.1 N NaOH mixture (2:1). Fill up with a Methanol / water mixture (1:1).

Determination:

Inject the standard solution until both the retention time and the pulse area of 3-Indole Butyric Acid remain steble. Then inject the sample solution.

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3-INDOLE BUTYRIC ACID Issue No 2 270686 B-37-5/5

Research Method

Phenol Phthalic Acid solution

Weigh out 100 mg into 60 ml of Alcohol 96% and replenish with water to 100 ml.

Thio Amide solution

Intermix 0.2 ml of a Thio Amide solution (4%) with a 1 ml mixture of 15 ml of 1M Sodium Hydroxide, 5 ml of water and 20 ml of Glycerol. Heat the solution in a water bath during 20 seconds and use immediately. Always prepare freshly.

Acetate buffer pH 3.5

Dissolve 25 grams of Ammonium Acetate in 25 ml of water. Add 38 ml of 7M Hydrochloric Acid. Make up to pH 3.5 by adding 2M Hydrochloric Acid or 5M Ammonia and replenish with water to 100 ml.

Iron Sulphate 1 ppm

Weigh out 0.864 gram of $FeNH_4(SO_4)_2.12H_2O$ into a 1000 ml volumetric flask. Dissolve in 25 ml of 2N Sulphuric Acid and replenish with water to 1000 ml. Take 1.0 ml of this solution and replenish with water to 100.0 ml.

Lead Nitrate 10 ppm

Weigh out 1.598 grams of Lead (II) Nitrate into a 1000 ml volumetric flask. Dissolve in water, then replenish with water to 1000 ml. Take 1.0 ml of this solution and replenish with water to 100.0 ml